CHO, BHK, 293 cells, Vero expressed
  interferon alpha-14C.
h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

              1. CMP-SA-PEG, $\alpha 2,8$-ST

h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
 j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
  when j-m (independently selected) is 2;
z = 1; n = 0; R = PEG.

FIG. 30J

CHO, BHK, 293 cells, Vero expressed
  Interferon alpha-14C.
a-g, j-m, r-u (independently selected) = 0 or 1;
h = 1 to 3; n, v-y = 0; z = 1.

         1. Sialidase
         2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1;
h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 30K

CHO, BHK, 293 cells, Vero expressed
   interferon alpha-14C.
h = 1 to 3;
 a-g, j-m, i (independently selected) = 0 or 1;
 r-u (independently selected) = 0 or 1;
 n, v-y = 0; z = 1.

1. CMP-SA, α2,8-ST

h = 1 to 3;
 a-g, i, r-u (independently selected) = 0 or 1;
 j-m (independently selected) = 0 to 40;
 z = 1; v-y, n = 0.

FIG. 30L

Insect cell or fungi expressed interferon alpha-14C.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.

1. GNT's 1 & 2, UDP-GlcNAc
2. Galactosyltransferase,
     UDP-Gal-linker-SA-CMP
3. ST3Gal3, transferrin

a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1;
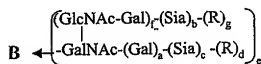z = 1; b, d, f, h, j-n, s, u, w, y = 0;
R = transferrin.

FIG. 30M

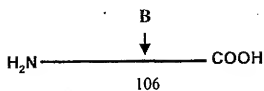Insect cell or fungi expressed interferon alpha-14C.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1; z = 1.

1. endoglycanase
2. Galactosyltransferase,
   UDP-Gal-linker-SA-CMP
3. ST3Gal3, transferrin

i (independently selected) = 0 or 1;
a-h, j-m, r-z = 0;
n = 1; R' = -Gal-linker-transferrin.

FIG. 30N

$$\text{H}_2\text{N} \underset{106}{\overset{\overset{\textstyle B}{\downarrow}}{\rule{0pt}{0pt}\text{———————}}} \text{COOH}$$

$$B \longleftarrow \left[\begin{array}{c} \text{(GlcNAc-Gal)}_f\text{-(Sia)}_b\text{-(R)}_g \\ | \\ \text{-GalNAc-(Gal)}_a\text{-(Sia)}_c\text{-(R)}_d \end{array} \right]_e$$

a-c, e, f (independently selected) = 0 or 1;
d, g = 0; R = polymer, glycoconjugate.

FIG. 30O

CHO, BHK, 293 cells, Vero expressed
IF-alpha (2a or 2b).
a-c (independently selected) = 0 or 1;
e = 1; d, f, g = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1

a-d (independently selected) = 0 or 1;
e = 1; b, f, g = 0; R = PEG.

FIG. 30P

Insect cell expressed interferon alpha (2a or 2b).
a, e (independently selected) = 0 or 1;
b, c, d, f, g = 0.

1. Galactosyltransferase, UDP-Gal
2. CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1;
b, f, g = 0; R = PEG.

FIG. 30Q

E. coli expressed IF-alpha (2a or 2b).
a-g = 0.

1. GalNAc Transferase,
   UDP-GalNAc-PEG

a-c, f, g = 0; d, e = 1; R = PEG.

FIG. 30R

NSO expressed IF-alpha (2a or 2b).
a (independently selected) = 0 or 1;
e = 1; b, c, d, f, g = 0

1. CMP-SA-levulinate, ST3Gal1
2. H_4N_2-PEG

a, c, d (independently selected) = 0 or 1;
e = 1; b, f, g = 0; R = PEG.

FIG. 30S

E. coli expressed IF-alpha (2a or 2b).
a-g = 0.

1. Endo-N-acetylgalatosamidase
   (synthetic enzyme),
   PEG-Gal-GalNAc-F

a, d, e = 1; b, c, f, g = 0; R = PEG.

FIG. 30T

E. coli expressed IF-alpha (2a or 2b).
a-g = 0.

1. GalNAc Transferase, UDP-GalNAc
2. sialyltransferase, CMP-SA-PEG

b, d = 0 or 1; e = 1; a, c, f, g = 0; R = PEG.

FIG. 30U

CHO, BHK, 293 cells, Vero expressed IF-alpha
(2a or 2b).
a-c, f (independently selected) = 0 or 1;
e = 1; d, g = 0

    1. Sialidase
    2. CMP-SA-PEG, ST3Gal1 and ST3Gal3

a-d, f, g (independently selected) = 0 or 1;
e = 1; R = PEG.

FIG. 30V

CHO, BHK, 293 cells, Vero expressed
  IF-alpha (2a or 2b).
a-c, f (independently selected) = 0 or 1;
e = 1; d, g = 0

    1. Sialidase
    2. CMP-SA-linker-SA-CMP,
     ,ST3Gal1
    3. ST3Gal3, transferrin

a-d, f (independently selected) = 0 or 1;
e = 1; R = transferrin; g = 0.

FIG. 30W

$$a\text{-}c, e \text{ (independently selected)} = 0 \text{ or } 1;$$
$$d = 0; \ R = \text{polymer, glycoconjugate.}$$

FIG. 30X

> CHO, BHK, 293 cells, Vero expressed
> interferon alpha-mucin fusion protein.
> a-c, e (independently selected) = 0 or 1; d = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1

> a-d, e (independently selected) = 0 or 1;
> R = PEG.

FIG. 30Y

> Insect cell expressed interferon alpha-mucin
> fusion protein.
> a, e (independently selected) = 0 or 1;
> b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

> a, d, e (independently selected) = 0 or 1;
> b, c = 0; R = PEG.

FIG. 30Z

E. coli expressed interferon alpha-mucin
  fusion protein.
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
 a, b = 0; R = PEG.

FIG. 30AA

B ← $\left[\begin{array}{c} (Sia)_b \\ | \\ \text{GalNAc-(Gal)}_a\text{-(Sia)}_c\text{- (R)}_d \end{array} \right]_e$

C ← (R')$_n$

a-c, e (independently selected) = 0 or 1;
d = 0; R = polymer, linker.

FIG. 30BB

E. coli expressed interferon alpha-mucin
  fusion protein.
a-e, n = 0.

↓ 1. GalNAc Transferase,
     UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1;
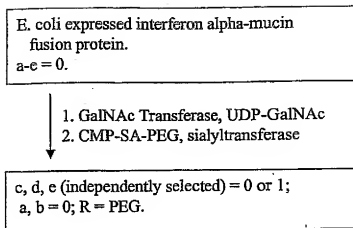a-c, n = 0; R = PEG.

FIG. 30CC

E. coli expressed interferon alpha-mucin fusion
protein.
a-e, n = 0.

↓ 1. GalNAc Transferase,
     UDP-GalNAc-linker-SA-CMP
  2. ST3Gal3, asialo-transferrin
  3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1;
a-c, n = 0; R = linker-transferrin.

FIG. 30DD

E. coli expressed Interferon alpha (no fusion).
a-e, n = 0.

1. NHS-CO-linker-SA-CMP
2. ST3Gal3, transferrin

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 30EE

$$\text{A} \leftarrow \begin{matrix} (\text{Fuc})_i \\ | \\ \text{GlcNAc} \\ | \\ (\text{R'})_n \end{matrix} \text{GlcNAc-Man} \left\{ \begin{matrix} \text{Man} \begin{bmatrix} [\text{GlcNAc-(Gal)}_a]_e - (\text{Sia})_j - (\text{R})_v \end{bmatrix}_r \\ [\text{GlcNAc-(Gal)}_b]_f - (\text{Sia})_k - (\text{R})_w \end{bmatrix}_s \\ \text{Man} \begin{bmatrix} [\text{GlcNAc-(Gal)}_c]_g - (\text{Sia})_l - (\text{R})_x \end{bmatrix}_t \\ [\text{GlcNAc-(Gal)}_d]_h - (\text{Sia})_m - (\text{R})_y \end{bmatrix}_u \end{matrix} \right\}_z$$

a-d, i, r-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1; R = polymer

FIG. 31A

CHO, BHK, 293 cells, Vero expressed IF-beta
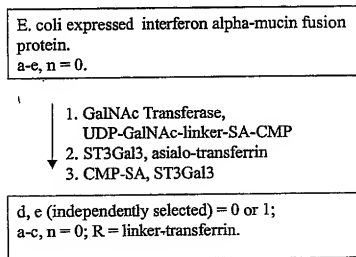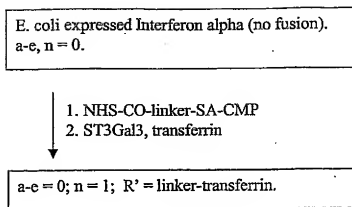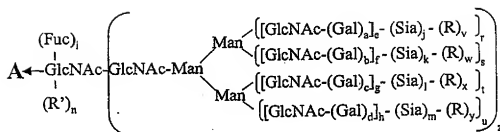h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

↓ 1. Sialidase
   2. CMP-SA-PEG, ST3Gal3

h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.

FIG. 31B

Insect cell expressed IF-beta
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.

↓ 1. GNT's 1&2, UDP-GlcNAc
   2. Galactosyltransferase, UDP-Gal
   2. CMP-SA-PEG, ST3Gal3,
      buffer, salt

b, d, f, h, k, m, n, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
z = 1; R = PEG.

FIG. 31C

Yeast expressed IF-beta
a-n = 0; z = 1;
r-y (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose or
polysaccharide.

    1. Endo-H
    2. Galactosyltransferase, UDP-Gal
    3.. CMP-SA-PEG, ST3Gal3

a-m, r-z= 0; n = 1; R' = -Gal-Sia-PEG.

FIG. 31D

CHO, BHK, 293 cells, Vero expressed IF-beta
h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

    1. CMP-SA-PEG, ST3Gal3

h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.

FIG. 31E

Insect cell expressed IF-beta
a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t
(independently selected) = 0 or 1; z = 1.

1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.

FIG. 31F

Yeast expressed IF-beta
a-n = 0; z = 1;
r-y (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose.

1. mannosidases
2. GNT's 1,2,4,5, UDP-GlcNAc
3. Galactosyltransferase, UDP-Gal
4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.

FIG. 31G

NSO expressed IF-beta
a-i, r-u (independently selected) = 0 or 1;
j-m, n, v-y = 0; z = 1.

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

a-i, j-m, r-y (independently selected) = 0 or 1;
n = 0; z = 1; R = PEG.

FIG. 31H

CHO, BHK, 293 cells, Vero expressed IF-beta
h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

1. CMP-SA-PEG, $\alpha2,8$-ST

h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
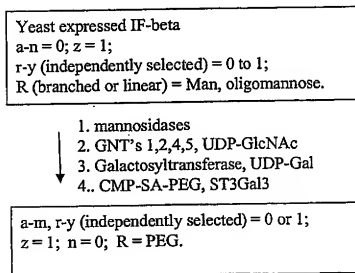   when j-m (independently selected) is 2;
z = 1; n = 0; R = PEG.

FIG. 31I

CHO, BHK, 293 cells, Vero expressed IF-beta
a-g, j-m, r-u (independently selected) = 0 or 1;
h = 1 to 3; n, v-y = 0; z = 1.

1. Sialidase
2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1;
h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 31J

CHO, BHK, 293 cells, Vero expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.

1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),
   ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n=0;
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31K

NSO expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0;
Sia (independently selected) = Sia or Gal.

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; R = PEG
n = 0; v-y (independently selected) = 1,
  when j-m (independently selected) is 1;
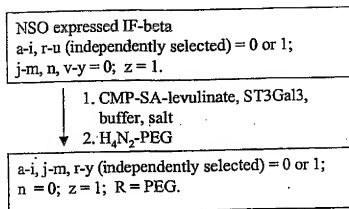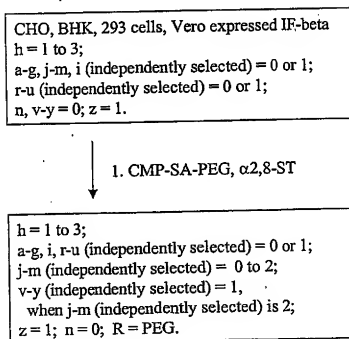
FIG. 31L

CHO, BHK, 293 cells, Vero expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
   ST3Gal3
3. CMP-SA, ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n = 0;
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31M

CHO, BHK, 293 cells, Vero expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n = 0;
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31N

CHO, BHK, 293 cells, Vero expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.

1. CMP-SA, $\alpha 2,8$-ST

a-d, i, r-u, z (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
n, v-y (independently selected) = 0.

FIG. 31O

$$A \leftarrow \begin{matrix} (Fuc)_i \\ | \\ GlcNAc \\ | \\ (R')_n \end{matrix} - GlcNAc-Man \left\{ \begin{matrix} Man \begin{matrix} [[GlcNAc-(Gal)_a]_e- (Sia)_j - (R)_v]_r \\ [[GlcNAc-(Gal)_b]_f- (Sia)_k - (R)_w]_s \end{matrix} \\ Man \begin{matrix} [[GlcNAc-(Gal)_c]_g- (Sia)_l - (R)_x]_t \\ [[GlcNAc-(Gal)_d]_h- (Sia)_m - (R)_y]_u \end{matrix} \end{matrix} \right\}_q \Big]_p$$

a-d, i, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = modifying group;
R' = H, glycosyl group, modifying group,
glycoconjugate.

FIG. 31P

Insect cell expressed Ifn-beta.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

    1. GNT's 1,2,4,5, UDP-GlcNAc
    2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
  when e-h (independently selected) is 1;
R = PEG.

FIG. 31Q

Yeast expressed Ifn-beta.
a-m = 0; q-y (independently selected) = 0 to 1;
p = 1;
R (branched or linear) = Man, oligomannose.

    1. Endoglycanase
    2. Galactosyltransferase, UDP-Gal
    3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.

FIG. 31R

CHO, BHK, 293 cells, Vero expressed Ifn-beta.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-linker-SA-CMP,
   ST3Gal3
2. ST3Gal3, desialylated transferrin.
3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1;
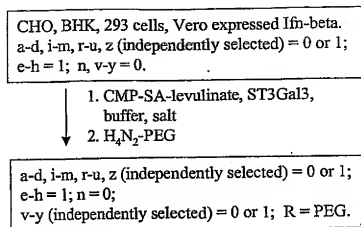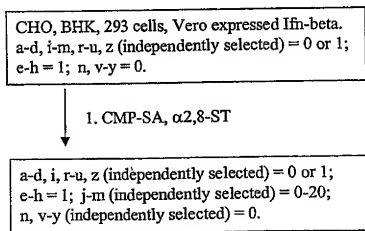p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-transferrin.

FIG. 31S

$$A \leftarrow (Fuc)_i \atop | \atop GlcNAc\text{-}GlcNAc\text{-}Man \left\{ \begin{matrix} Man \begin{matrix} [[GlcNAc\text{-}(Gal)_a]_e\text{-}(Sia)_j\text{-}(R)_v]_r \\ [[GlcNAc\text{-}(Gal)_b]_f\text{-}(Sia)_k\text{-}(R)_w]_s \end{matrix} \\ Man \begin{matrix} [[GlcNAc\text{-}(Gal)_c]_g\text{-}(Sia)_l\text{-}(R)_x]_t \\ [[GlcNAc\text{-}(Gal)_d]_h\text{-}(Sia)_m\text{-}(R)_y]_u \end{matrix} \end{matrix} \right\}_q$$

$$B \leftarrow [Glc\text{-}(Xyl)_n]_o$$

$$C \leftarrow [Fuc]_p$$
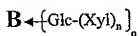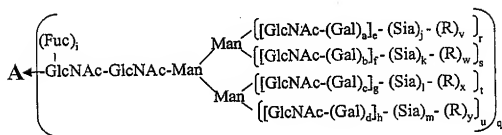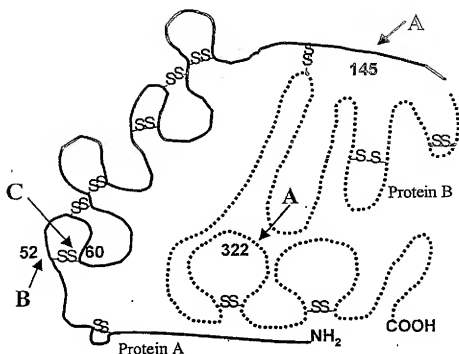
a-d, i, q-u (independently selected) = 0 or 1.
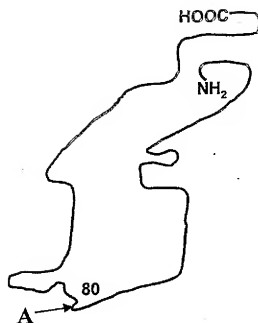o, p (independently selected) = 0 or 1.
e-h, n (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-y = 0;
R = modifying group, mannose, oligo-
mannose, Sia-Lewis X, Sia-Lewis A..

FIG. 32A

BHK expressed Factor VII or VIIa
a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

1. Sialidase
2. CMP-SA-PEG (16 mole eq),
   ST3Gal3

a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;
v, x, (independently selected) = 1,
when j, l (respectively, independently selected) is 1;
R = PEG.

FIG. 32B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa
a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

1. Sialidase
2. CMP-SA-PEG (1.2 mole eq),
   ST3Gal3
3. CMP-SA (8 mol eq), ST3Gal3

a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;
v or x, (independently selected) = 1,
 when j or l, (respectively, independently selected) is 1;
R = PEG.

FIG. 32C

```
NSO expressed Factor VII or VIIa
a--u (independently selected) = 0 or 1;
v-y = 0;  n = 0-4;
Sia (independently selected) = Sia or Gal.
```

1. Sialidase and α-galactosidase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

```
a-m, o-u (independently selected) = 0 or 1;
n = 0-4;  v-y (independently selected) = 1,
  when j-m (independently selected) is 1;
Sia = Sia;  R = PEG.
```

FIG. 32D

$$(Fuc)_i$$

$$A \longleftarrow -GlcNAc-GlcNAc-Man \begin{array}{c} Man \left[\begin{array}{l} [GlcNAc-(Gal)_a]_e - (Sia)_j - (R)_v \\ {[GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w} \end{array} \right]_s \\ Man \left[\begin{array}{l} [GlcNAc-(Gal)_c]_g - (Sia)_l - (R)_x \\ {[GlcNAc-(Gal)_d]_h - (Sia)_m - (R)_y} \end{array} \right]_u \end{array}$$

$$B \longleftarrow \begin{array}{c} (Sia)_o \\ | \\ -GalNAc-(Gal)_n-(Sia)_p-(R)_z \end{array} \Bigg]_q \qquad C \longleftarrow -Glc-(Xyl)_{aa}\Big]_{bb}$$

$$D \longleftarrow -Fuc \left[-(GlcNAc)_{cc}-(Gal)_{dd}-(Sia)_{ee} \right]_{ff} -(R)_{gg}$$
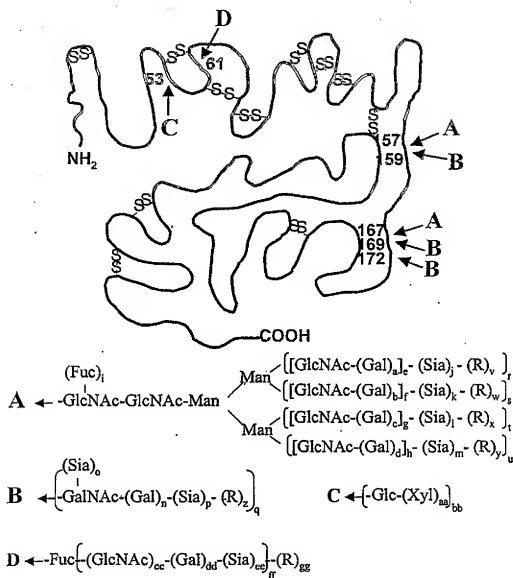
a-d, i, n-u (independently selected) = 0 or 1.

bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1.

e-h, aa (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-z = 0; R = modifying group, mannose, oligo-mannose.

FIG. 33A

CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently
  selected) = 0 or 1;
v-z, gg = 0.

1. Sialidase
2. CMP-SA-PEG, ST3Gal3

a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
  = 0 or 1;
o, p, z = 0;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
R = PEG.

FIG. 33B

CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, n, q = 1; e-h = 1 to 4;
 aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently
   selected) = 0 or 1;
v-z, gg = 0.

1. Sialidase
2. CMP-SA-PEG, ST3Gal3
3. ST3Gal1, CMP-SA

a-d, n, p, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) =
  0 or 1;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
o, z = 0; R = PEG.

FIG. 33C

```
CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, n, q, bb, cc, dd, ff = 1;  e-h, aa = 1 to 4;  ee, j-m, i,
o, p, r-u (independently selected) = 0 or 1;  v-z, gg = 0.
```

1. sialidase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA, ST3Gal3
4. CMP-SA-PEG, ST3Gal1

```
a-d, n, q = 1;  e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) =
  0 or 1; R = PEG;
o, v-y, gg = 0;
j-m, p, ee (independently selected) = 0 or 1, but when
p = 1, z = 1.
```

FIG. 33D

```
CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, q = 1;  e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently
  selected) = 0 or 1;
v-z, gg = 0.
```

CMP-SA-PEG, ST3Gal3

```
a-d, q = 1;  e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
  = 0 or 1;  R = PEG;
o, p, z = 0;  j-m, ee, v-y, gg (independently selected) =
  0 or 1.
```

FIG. 33E

CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently
  selected) = 0 or 1;
v-z, gg = 0.

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
  = 0 or 1;
o, p, z = 0; R = PEG;
j-m, ee, v-y, gg (independently selected) = 0 or 1.
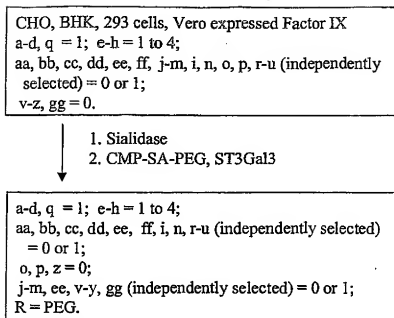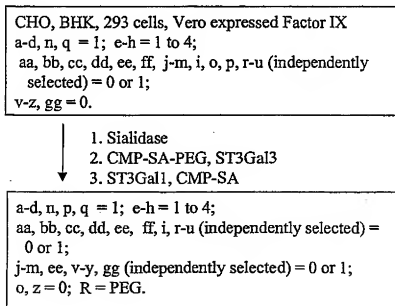
FIG. 33F

CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, n, q, bb, cc, dd, ff = 1;
e-h, aa = 1 to 4;
ee, j-m, i, o, p, r-u (independently selected) = 0 or 1;
  v-z, gg = 0.

1. CMP-SA-PEG, $\alpha 2,8$-ST

a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
  = 0 or 1;
o, p, z = 0; R= PEG;
j-m, ee (independently selected) = 0 to 2;
v-y, gg (independently selected) = 1, when j-m
  (independently selected) is 2;

FIG. 33G

FSHα

A   A

H_2N ——————↓——↓—— COOH
                  52   78

FSHβ

A   A

H_2N ——↓——↓————————————— COOH
         7   24

$$A \left\{ \begin{array}{c} (Fuc)_i \\ | \\ GlcNAc\text{-}GlcNAc\text{-}Man \\ | \end{array} \right. \quad \begin{array}{c} Man \left\langle \begin{array}{c} [[GlcNAc\text{-}(Gal)_a]_e\text{-}(Sia)_j\text{-}(R)_v]_r \\ [[GlcNAc\text{-}(Gal)_b]_f\text{-}(Sia)_k\text{-}(R)_w]_s \end{array} \right. \\ Man \left\langle \begin{array}{c} [[GlcNAc\text{-}(Gal)_c]_g\text{-}(Sia)_l\text{-}(R)_x]_t \\ [[GlcNAc\text{-}(Gal)_d]_h\text{-}(Sia)_m\text{-}(R)_y]_u \end{array} \right. \end{array} \right\}_q$$

a-d, i, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose.

FIG. 34A

CHO, BHK, 293 cells, Vero expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

　　　　　1. Sialidase
　　　　　2. CMP-SA-PEG (16 mol eq),
　　　　　　　ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
  when j-m (independently selected) is 1;
R = PEG.

FIG. 34B

CHO, BHK, 293 cells, Vero expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

　　　　　1. Sialidase
　　　　　2. CMP-SA-PEG (1.2 mol eq),
　　　　　　　ST3Gal3
　　　　　3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 34C

NSO expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.

         1. Sialidase and α-galactosidase
         2. Galactosyltransferase, UDP-Gal
         3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
  when j-m (independently selected) is 1;
R = PEG.

FIG. 34D

CHO, BHK, 293 cells, Vero expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

         1. Sialidase
         2. CMP-SA-PEG (16 mol eq),
            ST3Gal3
         3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 34E

CHO, BHK, 293 cells, Vero expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

    1. CMP-SA-levulinate, ST3Gal3,
       buffer, salt
    2. H$_4$N$_2$-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 34F

CHO, BHK, 293 cells, Vero expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

    1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
v-y (independently selected) = 0.

FIG. 34G

Insect cell expressed FSH.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

      1. GNT's 1,2,4,5, UDP-GlcNAc
      2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
  when e-h (independently selected) is 1;
R = PEG.

FIG. 34H

Yeast expressed FSH.
a-m = 0; q-y (independently selected) = 0 to 1;
p = 1;
R (branched or linear) = Man, oligomannose.

      1. Endoglycanase
      2. Galactosyltransferase, UDP-Gal
      3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.

FIG. 34I

CHO, BHK, 293 cells, Vero expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-linker-SA-CMP, ST3Gal3
2. ST3Gal1, desialylated chorionic
   gonadrophin (CG) produced in CHO.
3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
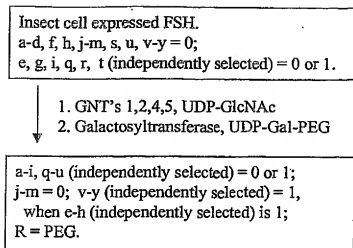R = linker-CG.

FIG. 34J

$$A \leftarrow \overset{(Fuc)_i}{\underset{|}{\text{-GlcNAc-GlcNAc-Man}}} \begin{array}{c} \text{Man} \\ \\ \text{Man} \end{array} \begin{array}{l} \Big[[\text{GlcNAc-(Gal)}_a]_e\text{- (Sia)}_j\text{- (R)}_v \Big]_r \\ \Big[[\text{GlcNAc-(Gal)}_b]_f\text{- (Sia)}_k\text{- (R)}_w \Big]_s \\ \Big[[\text{GlcNAc-(Gal)}_c]_g\text{- (Sia)}_l\text{- (R)}_x \Big]_t \\ \Big[[\text{GlcNAc-(Gal)}_d]_h\text{- (Sia)}_m\text{- (R)}_y \Big]_u \end{array}$$

$$B \leftarrow \overset{(Sia)_o}{\underset{|}{\Big[\text{-GalNAc-(Gal)}_n\text{-(Sia)}_p\text{- (R)}_z\Big]_q}}$$

a-d, i, n-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 to 20.
v-z = 0;
R = polymer.

FIG. 35A

CHO, BHK, 293 cells, Vero expressed EPO
a-g, n, q = 1; h = 1 to 3;
j-m, i, o, p (independently selected) = 0 or 1;
  r-u (independently selected) = 0 to1; v-z = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal3

a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
 r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.

FIG. 35B

Insect cell expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
 e, g, i, r, t (independently selected) = 0 or 1.

1. GNT's 1&2, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

b, d, f, h, k, m-q, s, u, w, y, z = 0;
a, c, e, g, i, r, t (independently selected)= 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
R = PEG.
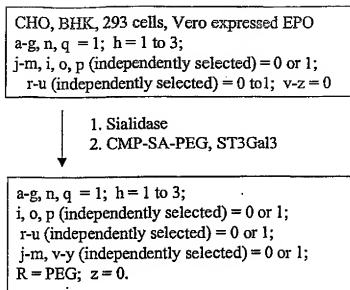
FIG. 35C

CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
 v-z = 0.

    1. sialidase
    2. Galactosyltransferase, UDP-Gal
    3. CMP-SA, ST3Gal3
    4. CMP-SA-PEG, ST3Gal1

a-h, n, q = 1;
 i-m, o, r-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.

FIG. 35D

CHO, BHK, 293 cells, Vero expressed EPO
a-g, n, q = 1; h = 1 to 3;
j-m, i, o, p (independently selected) = 0 or 1;
 r-u (independently selected) = 0 or 1;
 v-z = 0

    1. CMP-SA-PEG, ST3Gal3

a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
 r-u (independently selected) = 0 to 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.

FIG. 35E

Insect cell, yeast ·or fungi expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

    1. GNT's 1, 2 & 5, UDP-GlcNAc
    2. Galactosyltransferase, UDP-Gal-PEG

a-c, e-g, i, r-t, v-x (independently selected) =
  0 or 1;
d, h, j-q, u, y, z = 0;   R = PEG.

FIG. 35F

Insect cell, yeast or fungi expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

    1. GNT's 1, 2 & 5, UDP-GlcNAc
    2. Galactosidase (synthetic enzyme),
       PEG-Gal-F.

a-c, e-g, n, q-t, v-x, z (independently selected) =
  0 or 1;
d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 35G

Insect cell, yeast or fungi expressed EPO
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

1. GNT-1, UDP-GlcNAc-PEG

e, i, r, v (independently selected) = 0 or 1;
a-h, j-q, s-u, w-z = 0; R = PEG.

FIG. 35H

Insect cell, yeast or fungi expressed EPO
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

1. GNT-1, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG

a, e, i, r, v (independently selected) = 0 or 1;
b-d, f-h, j-q, s-u, w-z = 0;   R = PEG.

FIG. 35I

Insect cell, yeast or fungi expressed EPO
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

    1. GNT-1, UDP-GlcNAc
    2. Galactosyltransferase, UDP-Gal
    3. ST3Gal3, CMP-SA-PEG

a, e, i, j, r, v (independently selected) = 0 or 1;
b-d, f-h, k-q, s-u, w-z = 0;   R = PEG.

FIG. 35J

Insect cell, yeast or fungi expressed EPO
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

    1. GNT's 1, 2 & 5, UDP-GlcNAc
    2. Galactosyltransferase, UDP-Gal
    3. ST3Gal3, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected)
   = 0 or 1;
d, h, m-q, u, y, z = 0; R = PEG.

FIG. 35K

Insect cell, yeast or fungi expressed EPO
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.

1. GNT's 1, 2 & 5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal
3. α2,6-sialyltransferase, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected)
  = 0 or 1;
d, h, m-q, u, y, z = 0;   R = PEG.

FIG. 35L

CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
v-z = 0.

1. sialidase
2. CMP-SA, ST3Gal3
3. CMP-SA-PEG, ST3Gal1

a-h, q, i-o, r-u (independently selected)
  = 0 or 1;
v-y = 0; p, z = 0 or 1;   R = PEG.

FIG. 35M

CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
    v-z = 0.

1. CMP-SA-PEG, ST3Gal3

a-h, i-o, q-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1;   R = PEG.
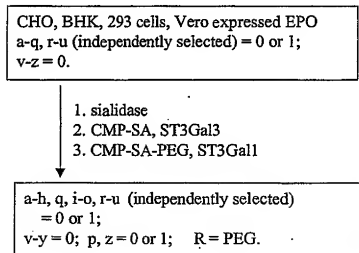
FIG. 35N

CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
    v-z = 0.

1. CMP-SA-PEG, α2,8-sialyltransferase

a-h, i-o, q-u (independently selected) = 0 or 1;
v-y = 0;   p, z = 0 or 1;   R = SA-PEG.
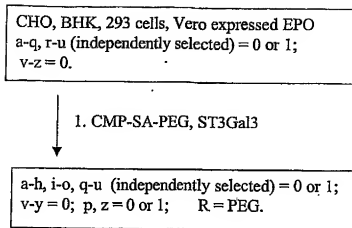
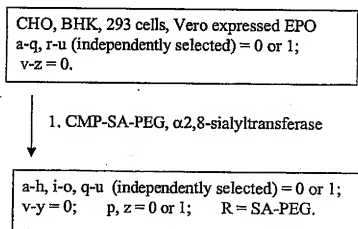FIG. 35O

CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
  v-z = 0.

1. CMP-SA-PEG, $\alpha 2,8$-sialyltransferase

a-h, i-o, p-u, v-z (independently selected)
  = 0 or 1;
R = SA-PEG.

FIG. 35P

yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
a-m, n-q, s, w, z = 0; R = (Man)$_n$
  where n = 1-5, linear or branched.

1. mannosidases
2. GNT-1, UDP-GlcNAc
3. galactosyltransferase, UDP-Gal
4. ST3Gal3, CMP-SA-PEG

a, e, j, r, v (independently selected) = 0 or 1;
  b-d, f-i, k-q, s-u, w-z = 0;   R = PEG.

FIG. 35Q

yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
a-m, n-q, s, w, z = 0; R = $(Man)_n$
   where n = 1-5, linear or branched.

$\downarrow$ 1. mannosidases
   2. GNT-1, UDP-GlcNAc-PEG

e, r, v (independently selected) = 0 or 1;
a-h, i-q, s-u, w-z = 0;.   R = PEG.

FIG. 35R

yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
a-m, n-q, s, w, z = 0; R = $(Man)_n$
   where n = 1-5, linear or branched.

$\downarrow$ 1. mannosidase-I
   2. GNT-1, UDP-GlcNAc
   3. galactosyltransferase, UDP-Gal
   4. ST3Gal3, CMP-SA-PEG

a, e, j, r, t-u, v, x, y (independently selected)
   = 0 or 1;
 b-d, f-i, k-q, s, w, z = 0;
 $(R)_v$ = PEG; $(R)_x$ and $(R)_y$ = Man.

FIG. 35S

$$A \leftarrow \text{-GlcNAc-GlcNAc-Man} \begin{cases} \text{(R'')}_i \\ \text{(R')}_{cc} \end{cases}$$

a-d, i, n-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 to 20.
v-z = 0;   aa, bb = 1; cc = 0;
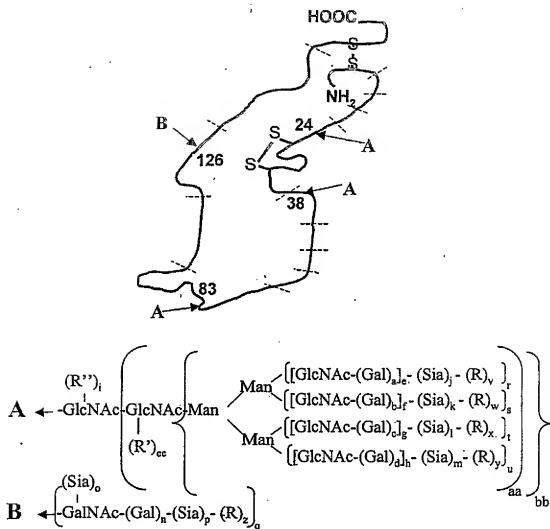R = polymer;   R'' and R' = sugar-polymer or Fuc.

FIG. 35T

yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
cc, a-m, n-q, s, w, z = 0;
aa, bb = 1;
R = (Man)$_n$ where n = 1-100, linear or branched.

1. endo-H
2. galactosyltransferase, UDP-Gal-PEG

i (independently selected) = 0 or 1;
aa, bb, cc, a-h, j-z = 0;   R" = Gal-PEG.

FIG. 35U

yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
cc, a-m, n-q, s, w, z = 0;   aa, bb = 1;
R = (Man)$_n$ where n = 1-100, linear or branched.

1. endo-H
2. galactosyltransferase, UDP-Gal
3. ST3Gal3, CMP-SA-PEG

i (independently selected) = 0 or 1;
aa, bb, cc, a-h, j-z = 0;   R" = Gal-SA-PEG.

FIG. 35V

Insect cell expressed EPO
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1;
aa = 1;   R" = Fuc.

1. mannosidases
2. galactosyltransferase, UDP-Gal-PEG

cc, e, i, r, v (independently selected) = 0 or 1;
bb, a-h, j-q, s-u, w-z = 0; aa = 1; R' = Gal-PEG.

FIG. 35W

$$A \leftarrow \overset{(Fuc)_i}{\underset{|}{-GlcNAc-GlcNAc-Man}} \begin{array}{l} Man \left\langle \begin{array}{l} \left[[GlcNAc-(Gal)_a]_e- (Sia)_j - (R)_v\right]_r \\ \left[[GlcNAc-(Gal)_b]_f- (Sia)_k - (R)_w\right]_s \end{array} \right. \\ Man \left\langle \begin{array}{l} \left[[GlcNAc-(Gal)_c]_g- (Sia)_l - (R)_x\right]_t \\ \left[[GlcNAc-(Gal)_d]_h- (Sia)_m - (R)_y\right]_u \end{array} \right. \end{array}$$

$$B \leftarrow \left[\overset{(Sia)_o}{\underset{|}{-GalNAc-(Gal)_n-(Sia)_p- (R)_z}}\right]_q$$
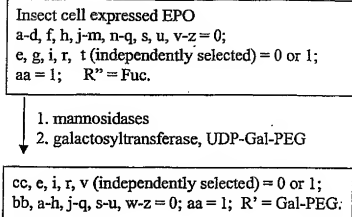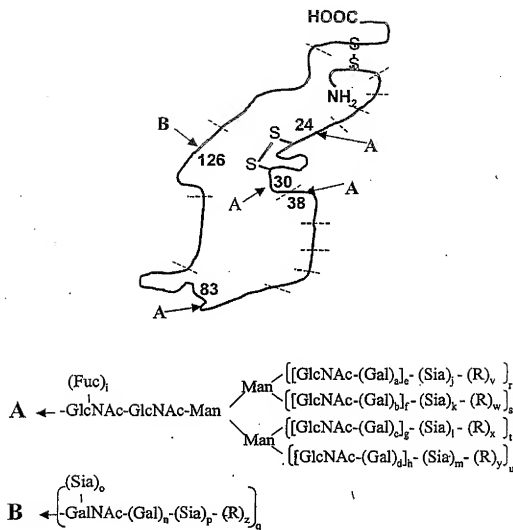
a-d, i, n-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 to 20.
v-z = 0;
R = polymer.

FIG. 35X

NSO expressed NESP
q = 1; a-i, n, r-u (independently selected) = 0
or 1; j-m, o, p, v-z = 0

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

q = 1; a-i, j-n, r-y (independently selected) =
   0 or 1;
o, p, z = 0; R = PEG.

FIG. 35Y

CHO, BHK, 293 cells, Vero expressed NESP
a-g, n, q = 1; h = 1 to 3;
j-m, i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
v-z = 0

1. CMP-SA-PEG, α2,8-ST

a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
   when j-m (independently selected) is 2;
R = PEG; z = 0.

FIG. 35Z

CHO, BHK, 293 cells, Vero expressed NESP
a-g, n, q = 1; h = 1 to 3;
j-m, i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to1; v-z = 0

↓ 1 CMP-SA, poly-α2,8-ST

a-g, n, q = 1; h = 1 to 3;
i, j-m, o, p, r-u, (independently selected) = 0 or 1;
v-z (independently selected) = 0-40; R = Sia.
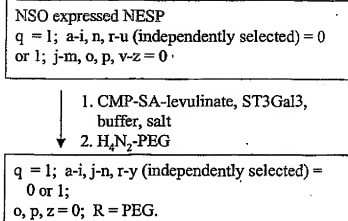
FIG. 35AA

FIG. 36A

CHO, BHK, 293 cells, Vero expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
   ST3Gal3

a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
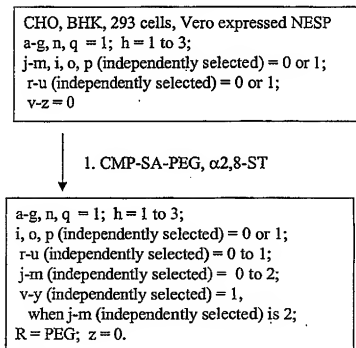v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
R = PEG.

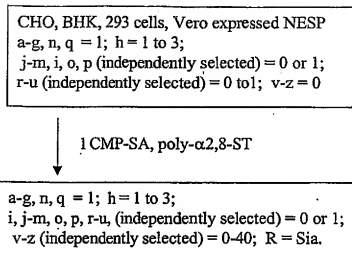**FIG. 36B**

CHO, BHK, 293 cells, Vero expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),
   ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3 &
   ST3Gal1

a-d, i-m, p-u, aa (independently selected) = 0 or 1;
o, z = 0; n, e-h = 1;
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 36C

NSO expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.

　　　　1. Sialidase and α-galactosidase
　　　　2. CMP-SA, ST3Gal3
　　　　2. CMP-SA-PEG, ST3Gal1

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1;
n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

FIG. 36D

CHO, BHK, 293 cells, Vero expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

　　　　1. Sialidase
　　　　2. CMP-SA-PEG (16 mol eq),
　　　　　　ST3Gal3
　　　　3. CMP-SA, ST3Gal3

a-d, i-m, q-y, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1; R = PEG.

FIG. 36E

CHO, BHK, 293 cells, Vero expressed   GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

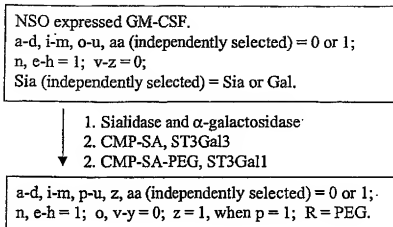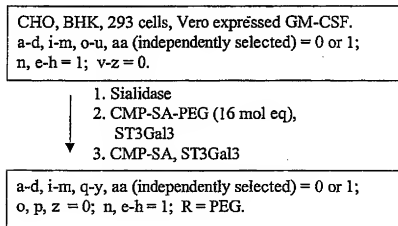a-d, i-m, o-y, aa (independently selected) = 0 or 1;
z = 0; n, e-h = 1; R = PEG.

FIG. 36F

CHO, BHK, 293 cells, Vero expressed GMCSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. CMP-SA, α2,8-ST

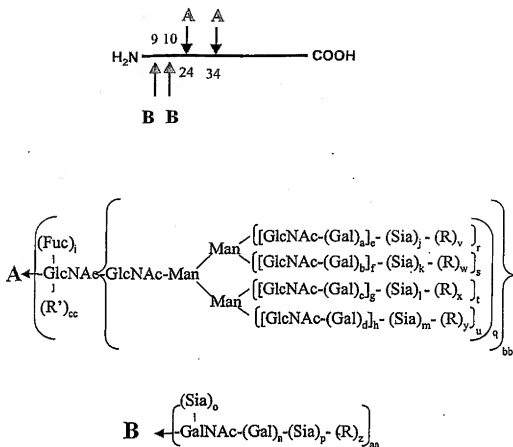a-d, i, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; j-m (independently selected) = 0-20;
v-z (independently selected) = 0.

FIG. 36G

a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = modifying group, mannose, oligo-mannose.
R' = H, glycosyl residue, modifying group. glycoconjugate.

FIG. 36H

Insect cell expressed GM-CSF.
a-d, f, h, j-m, o, p, s, u, v-z = 0;
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.

    1. GNT's 1,2,4,5, UDP-GlcNAc
    2. Galactosyltransferase, UDP-Gal-PEG

a-i, n, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
  when e-h (independently selected) is 1;
R = PEG.

FIG. 36I

Yeast expressed GM-CSF.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.

    1. Endoglycanase
    2. mannosidase (if aa = 1).
    3. Galactosyltransferase, UDP-Gal-PEG

a-p, r-z, aa, bb = 0;
q, cc (independently selected) = 0 or 1;
R' = -Gal-PEG.

FIG. 36J

CHO, BHK, 293 cells, Vero expressed GM-CSF.
a--m, o-u, aa, bb (independently selected) = 0 or 1;
n, v-z, cc = 0.

1. sialidase
2. CMP-SA, ST3Gal3
2. CMP-SA-linker-SA-CMP, ST3Gal1
3. ST3Gal3, transferrin

a--m, p-u, z, aa (independently selected) = 0 or 1;
o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 36K
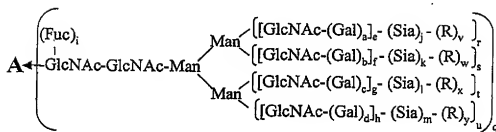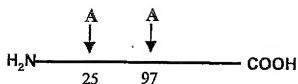
FIG. 37A

CHO, BHK, 293 cells, Vero expressed IF-gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

         1. Sialidase
         2. CMP-SA-PEG (16 mol eq),
            ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
R = PEG.

FIG. 37B

CHO, BHK, 293 cells, Vero expressed IF-gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

         1. Sialidase
         2. CMP-SA-PEG (1.2 mol eq),
            ST3Gal3
         3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
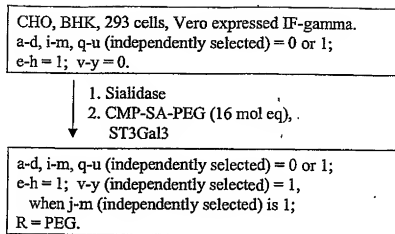
FIG. 37C

NSO expressed Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
  when j-m (independently selected) is 1;
R = PEG.

FIG. 37D

CHO, BHK, 293 cells, Vero expressed
  Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
   ST3Gal3
3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 37E

CHO, BHK, 293 cells, Vero expressed
    Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-levulinate, ST3Gal3,
2. H_4N_2-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 37F

CHO, BHK, 293 cells, Vero expressed
    Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
v-y (independently selected) = 0.

FIG. 37G

$$\text{H}_2\text{N} \underset{25 \quad 97}{\overset{A \qquad A}{\rule{6cm}{0.4pt}}} \text{COOH}$$

$$A \leftarrow \begin{array}{c} (\text{Fuc})_i \\ | \\ \text{GlcNAc} \\ | \\ (\text{R'})_n \end{array} \text{GlcNAc-Man} \begin{array}{l} \text{Man} \left\{ \begin{array}{l} \left[\text{GlcNAc-(Gal)}_a\right]_e - (\text{Sia})_j - (\text{R})_v \\ \left[\text{GlcNAc-(Gal)}_b\right]_f - (\text{Sia})_k - (\text{R})_w \end{array} \right\}_r \\ \text{Man} \left\{ \begin{array}{l} \left[\text{GlcNAc-(Gal)}_c\right]_g - (\text{Sia})_l - (\text{R})_x \\ \left[\text{GlcNAc-(Gal)}_d\right]_h - (\text{Sia})_m - (\text{R})_y \end{array} \right\}_t \end{array} \right\}_p$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
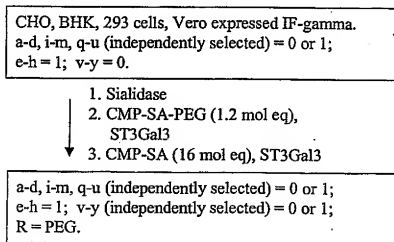j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 37H

Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
  when e-h (independently selected) is 1;
R = PEG.

FIG. 37I

Yeast expressed IF-gamma.
a-m = 0; q-y (independently selected) = 0 to 1; p = 1;
R (branched or linear) = Man, oligomannose.

1. Endoglycanase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.

FIG. 37J

CHO, BHK, 293 cells, Vero expressed IF-gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

   1. CMP-SA-linker-Gal-UDP, ST3Gal3
   2. Galactosyltransferase, transferrin treated
      with endoglycanase.

a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-transferrin.

FIG. 37K

CHO, BHK, 293 cells, Vero expressed
  Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1; n, v-y = 0.

   1. CMP-SA-PEG,
     ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1;
n, v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 37L
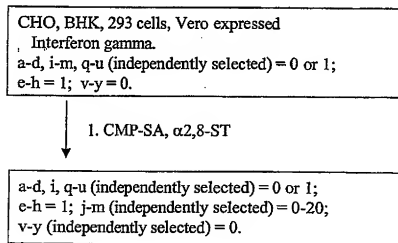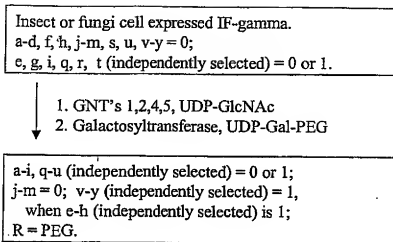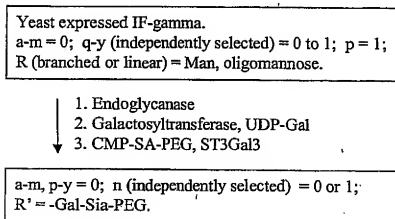
Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

    1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, q (independently selected) = 0 or 1;
p = 1; v, x (independently selected) = 1,
  when e, g (independently selected) is 1;
R = PEG.

FIG. 37M

CHO, BHK, 293 cells, Vero expressed
  Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

    1 . CMP-SA-PEG, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-2;
v-y (independently selected) = 1,
  when j-m (independently selected) = 2;
R = PEG.

FIG. 37N

$$A \begin{pmatrix} \text{(Fuc)}_i \\ | \\ \text{GlcNAc-GlcNAc-Man} \end{pmatrix} \begin{matrix} \text{Man} \begin{bmatrix} [\text{GlcNAc-(Gal)}_a]_e\text{- (Sia)}_j\text{- (R)}_v \end{bmatrix}_r \\ [\text{GlcNAc-(Gal)}_b]_f\text{- (Sia)}_k\text{- (R)}_w \end{bmatrix}_s \\ \text{Man} \begin{bmatrix} [\text{GlcNAc-(Gal)}_c]_g\text{- (Sia)}_l\text{- (R)}_x \end{bmatrix}_t \\ [\text{GlcNAc-(Gal)}_d]_h\text{- (Sia)}_m\text{- (R)}_y \end{bmatrix}_u \end{matrix}_q$$

a-d, i, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = polymer.

FIG. 38A

CHO, BHK, 293 cells, Vero or transgenic animal
   expressed α_1 antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

    1. Sialidase
    2. CMP-SA-PEG (16 mol eq),
       ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
R = PEG.

FIG. 38B

CHO, BHK, 293 cells, Vero or transgenic
   animal expressed α_1 antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

    1. Sialidase
    2. CMP-SA-PEG (1.2 mol eq),
       ST3Gal3
    3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 38C

CHO, BHK, 293 cells, Vero or transgenic animal
    expressed alpha-1 antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

        1. Sialidase
        2. CMP-SA-PEG (16 mol eq),
            ST3Gal3
        3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 38D

CHO, BHK, 293 cells, Vero or transgenic animal
    expressed α_1-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

        1. CMP-SA-levulinate, ST3Gal3,
            buffer, salt
        2. H_4N_2-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 38E

CHO, BHK, 293 cells, Vero expressed α_1-antitrypsin.
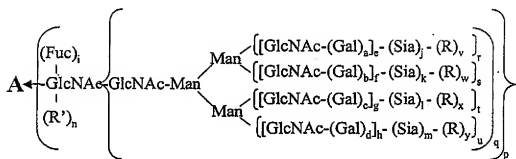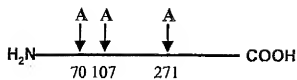a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1;
j-m (independently selected) = 0-20;
v-y (independently selected) = 0.

FIG. 38F

A A   A
          ↓ ↓   ↓
H₂N ─────────────────── COOH
          70 107   271

$$A \leftarrow \begin{Bmatrix} (Fuc)_i \\ | \\ GlcNAc-GlcNAc-Man \\ | \\ (R')_n \end{Bmatrix} \begin{Bmatrix} Man \begin{bmatrix} [GlcNAc-(Gal)_a]_e - (Sia)_j - (R)_v \\ [GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w \end{bmatrix}_s^r \\ Man \begin{bmatrix} [GlcNAc-(Gal)_c]_g - (Sia)_l - (R)_x \\ [GlcNAc-(Gal)_d]_h - (Sia)_m - (R)_y \end{bmatrix}_u^t \end{Bmatrix}_p$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
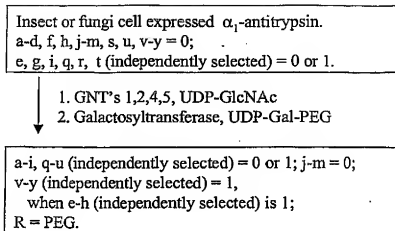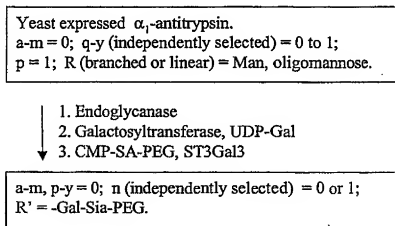R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 38G

Insect or fungi cell expressed α_1-antitrypsin.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-m = 0;
v-y (independently selected) = 1,
  when e-h (independently selected) is 1;
R = PEG.

FIG. 38H

Yeast expressed α_1-antitrypsin.
a-m = 0; q-y (independently selected) = 0 to 1;
p = 1; R (branched or linear) = Man, oligomannose.

1. Endoglycanase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;
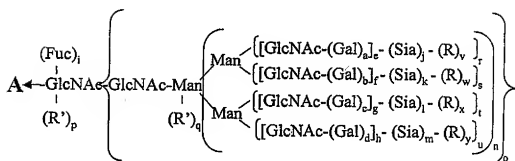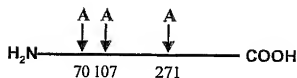R' = -Gal-Sia-PEG.

FIG. 38I

CHO, BHK, 293 cells, Vero expressed α_1-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

    1. CMP-SA-linker-Gal-UDP,
       ST3Gal3
    2. Galactosyltransferase, transferrin treated
       with endoglycanase

a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-transferrin.

FIG. 38J

a-d, i, n-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 to 20.
R = polymer;
R', R" (independently selected) = sugar, glycoconjugate.

FIG. 38K

Yeast expressed alpha-1 antitrypsin.
a-h, i-m, p, q = 0;
R (independently selected) = mannose, oligomannose,
polymannose;
r-u, v-y (independently selected) = 0 or 1;   n, o = 1.

→ 1. endoglycanase
▼ 2. Galactosyltransferase, UDP-Gal-PEG

a-h, i-o, q, r-u, v-y = 0; p = 1.
R" = Gal-PEG.

FIG. 38L

Plant expressed alpha-1 antitrypsin.
a-d, f, h, j- m, s, u , v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1;
n =1; R' = xylose

↓ 1. hexosaminidase,
   2. alpha mannosidase and xylosidase
   3. GlcNAc transferase, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u , v-y = 0;
e, g, i, r, t (independently selected) = 0;
q = 1; R' = GlcNAc-PEG.

FIG. 38M
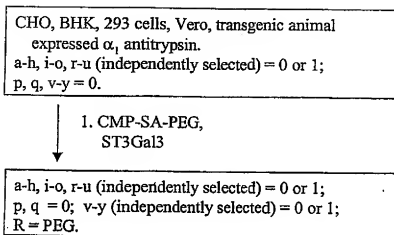
CHO, BHK, 293 cells, Vero, transgenic animal
  expressed α_1 antitrypsin.
a-h, i-o, r-u (independently selected) = 0 or 1;
p, q, v-y = 0.

                1. CMP-SA-PEG,
                   ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1;
p, q = 0; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 38N